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The Agricultural Chemical Society of Japan.

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The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Chemische Untersuchungen über das Glukomannan aus "Konjak".

V. Mitteilung. Methylierung des Glukomannans.

Von

Kitsuji Nishida und Hideo Hashima

(Eingegangen am 26 Juni 1931)

Der Methylierungsversuch gibt die Aufklärung über das Konstitutionsproblem des Glukomannans und durch ihn wird ein gewisser eindeutiger Abschluss erreicht. Zunächst wurden 10 g. Glukomannan mit 200 ccm. 15%-iger Natronlauge und 96 g. Dimethylsulfat methyliert. Nach 6-mal Methylierung betrug der Methoxylgehalt 34 %. Durch weitere Methylierungen, unter Verwendung von 20 g. Glukomannan, 30 ccm. 30%iger Natronlauge und 288 g. Dimethylsulfat für jeden Methylierungsversuch, war dann der Methoxylgehalt ein höhere geworden und 12～14 malige Methylierung diesen Wert 41.5% hatte erreichen lassen.

Bei der Methylierung von 2 g. methylierten Glukomannans (41.6% Methoxyl) mit 50 ccm. Methyljodid und 36 g. Silberoxyd erreichte der Wert nicht über 41.6% OCH_3. Wir müssen hieraus den Schluss ziehen, dass das Glukomannan bei der Methylierung mit Dimethylsulfat und Alkali oder mit Methyljodid und Silberoxyd gleichviel Methoxyl liefert wie die Cellulose und das Lichenin, d. h. dass er nicht über 42% zunehmen kann, und dass die Methylierung beim Arbeiten mit 30%iger Natronlauge und bei niedrigerer Temperatur leichter ist.

Das Methylglukomannan (OCH_3 41.6%) ist in kaltem Wasser kolloidal lösbar, in heissem Wasser dagegen tritt wieder Ausflockung ein, wie der Methyläther der Cellulose, des Lichenin und der Stärke. Es löst sich in Chloroform, Bromoform, Alkohol, Aceton, Eisessig und Essigester, doch haben wir eine Molekulargewichtsbestimmung nicht ausgeführt.

Die Hydrolyse des Glukomannanäthers führt zu methylierten Hexosen, und hat keine Abspaltung von Methylgruppen im Gefolge. Zu diesem Zweck wurden 29.8517 g. methyliertes Glukomannan (OCH_3 41.5 %) mit 1%iger methylalkoholischer Salzsäure in Bonbenröhren zu den methylierten Methylglukosiden und -mannosiden aufgespalten. Das Spaltungsprodukt wurde mit Silberkarbonat neutralisiert und es wurden nach dem Trocknen im Hochvakuum bei der fraktionierten Destillation fünf Fraktionen aufgefangen. Die ersten drei Fraktionen zeigten ein fast farbloses, die späteren ein schwach gelbliches Aussehen, und alle färbten beim Stehen in langer Zeit nach. Die Analyse ergaben.

	Sdp. (°C)	OCH_3 %		n_D^{15}	$[\alpha]_D^{20}$	
					in Wasser	in Methylalkohol
I Fraktion	124~125	50.83	50.11	—	—	—
II. Fraktion	125~127	51.10	50.74	1.4604	+43.8°	+45.6°
III. Fraktion	127~129	48.68	48.11	1.4587	+42.3°	+52.8°
IV-A. Fraktion	126~128	47.96	48.57	1.4597	+43.0°+42.5°	—
IV-B. Fraktion	128~139	47.16	47.08	1.4626	+45.9°	+56.6°

Aus diesen Versuchen geht also hervor, dass die Konstanten der Fraktionen, die aus methyliertem Glukomannan einerseits, aus Methylcellulose und -lichenin anderseits durch Spaltung gewonnen worden sind, eine voreinander erheblich abweichende Zusammensetzung zu haben scheinen, da das Glukomannan aus 2 Mol Mannose und 1 Mol Glukose besteht und die Spaltprodukte müssen Trimethylmannosid und -glukosid enthalten.

Die I. und II. Fraktion stehen im Methoxylgehalt ca. 1.1% unter denjenigen, welche sich für ein Trimethylmethylmannosid oder -glukosid aussprechen lassen wurden (52.6%); es muss darin kleine Mengen Dimethylmethylmannosid oder -glukosid (41.8%), das in der III.-IV. Fraktion überwiegt, enthalten sein. Der Methoxylgehalt der II. Fraktion war sehr gleichmässig, aber Brechungsindex war etwas hoch, und die spezifische Drehung war klein, verglichen nicht mit denjenigen, die bei Methylcellulose und -lichenin durch Abbau erhalten werden.

Die II. Fraktion wurde mit wässeriger Salzsäure verseift und dabei schied sich nicht die kristallisierte 2, 3, 6-Trimethylglukose (41.89% OCH_3) nach langem Stehen wie diejenige, die aus der ersten Fraktion von Methylbaumwolle und Methylolichenin abgebaut wurde, ab. Dieser Sirup erwies:

%OCH_3 40.49 ; 40.49.

Es muss darin eine Spur Dimethylhexose (29.81% OCH_3) enthalten sein. Wir haben diesen Sirup mit Salpetersäure (spez. Gew. 1.20) oxydiert und erhielten Trimethylzuckersäurelakton oder -mannozuckersäurelakton mit folgenden Eigenschaften:

OCH_3-Gehalt gef. 37.15; 36.89%
Berechnet für Trimethylzuckersäurelakton 39.74%
Verbrauch von *N*/10-Natronlauge. 10.00 ccm. (Substanz 0.116 g.)

Aus diesem Ergebnis geht also hervor, dass die I. und II. Fraktion 2, 3, 4-Trimethylhexosid waren mit kleine Mengen Dimethylhexosid und nicht 2, 3, 6-Trimethylmethylhexosid, und mit grosser Wahrscheinlichkeit handelte es sich um 2, 3, 4-Trimethylglukosid und nicht Mannosid. Die III, IV-A und -B Fraktion wurden auch wie die erste Fraktion mit Salzsäure verseift, dabei wir Trimethylhexose erhielten, die nach langem Stehen keine Kristalle

abschied. Die Analyse ergab:

Fraktion	%OCH_3		$[\alpha]_D^{20}$
III.	38.04	38.01	+36° in Wasser
IV–A	38.73	38.46	+18.5° in Chloroform
IV–B	36.26	36.38	+11.6° in Chloroform

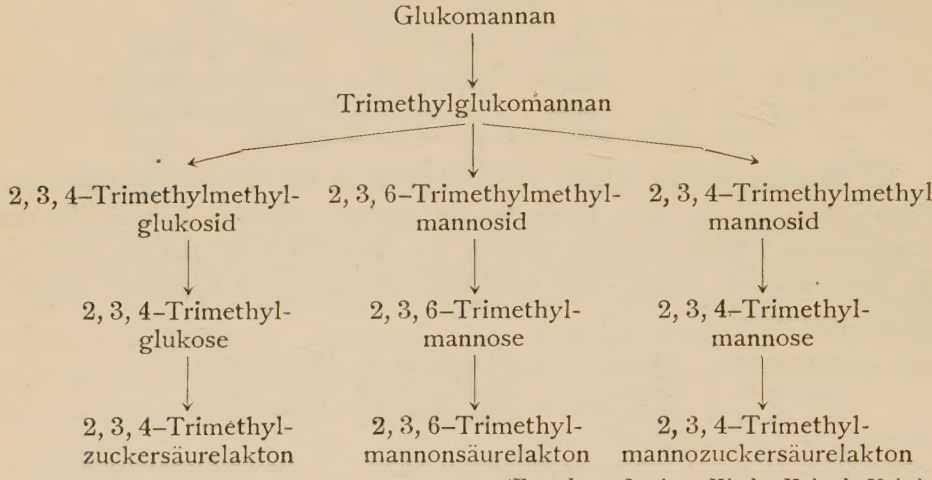
Im Methoxylgehalt stehen diese Fraktionen ca. 3.3～5.6% unter der Trimethylhexose; es muss darin bereits geringe Mengen Dimethylhexose enthalten sein. Dies Fraktion wurden wie oben mit Salpetersäure oxydiert, und dabei wurden Mono– und Dikarbonsäurelakton mit folgenden Eigenschaften gewonnen.

Aus der III. Fraktion OCH_3-Gehalt gef. 36.12; 35.78
Aus der IV–A Fraktion OCH_3-Gehalt gef. 35.18; 35.28
Aus der IV–B Fraktion OCH_3-Gehalt gef. 33.89; 33.76

Fraktion	Substanz	Verbrauch von *N*/10-Natronlauge	Fraktion	Substanz	Verbrauch von *N*/10-Natronlauge
III. Fraktion	0.1071 g.	7.52ccm	IV–B Fraktion	0.1180	9.16
IV–A Fraktion	0.1762	9.98	IV–B Fraktion	0.2631	19.33

Aus hier nahm in der III. und der IV–B Fraktion die Acidität zu, während der Säuregehalt in der IV–A Fraktion zur Hälfte zurückging. Bei all diesem Säurelakton handelte es sich selbstverständlich um Mischungen, aus denen die erste (aus der III Fraktion) eine Lösung von Trimethylzuckersäurelakton mit kleinen Mengen Trimethylmannonsäurelakton, die zweite (aus der IV–A Fraktion) ein Sirup von Trimethylmannonsäurelakton mit einer Spur Tri– und Dimethylmannozuckersürelakton, die dritte (aus der IV–B Fraktion) eine Mischung von Tri– und Dimethylmannozuckersäurelakton waren. Bei 2, 3, 6–Trimethylmethylglukosid Fraktion finden sich nach Verseifung mit Salzsäure bekanntlich Kristalle von 2, 3, 6–Trimethylglukose, die mit Salpetersäure zu 2, 3, 6–Trimethylglukonsäurelakton (Monokarbonsäurelakton) oxydieren müssen. Der grössere Teil aus der IV–A Fraktion war 2, 3, 6–Trimethylmethylhexosid, aber seine Eigenschaften und das Ergebnis ihrer Analyse zeigte nicht so grosse Aehnlichkeit, dass es sich hier um 2, 3, 6–Trimethylmethylglukosid handeln konnte; der Versuch, in welchem aus methyliertem Glukomannan durch Spaltung keine Kristalle gewonnen wurden, bildete also auch eine Stütze für die Abwesenheit der 2, 3, 6-Trimethylglukose. Da die Trimethylglukose und –mannose mit Phenylhydrazin kein Phenylosazon liefert, so muss am Kohlenstoffatom 2 eine Methoxylgruppe sitzen. Als Gesamtergebnis der im vorstehenden skizzierten Untersuchungen auf dem

Gebiete des Methylglukomannans folgt, dass das Spaltprodukt auf die Weise gewonnen wurde, wie sie das nachstehende Schema zeigt:



(Forstchem. Institut, Kiushu Kaiserl. Univ.)

Untersuchung über das ätherische Oel aus *Podocarpus macrophylla Don.* III.

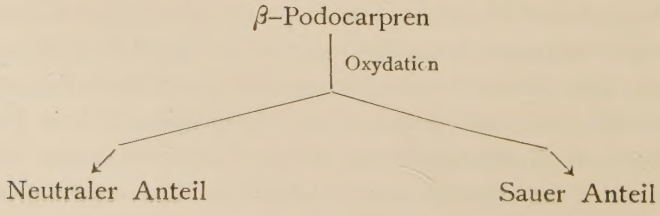
Mitteilung. Ueber die oxydierenden Produkten der neuen Diterpene (*α*– und *β*–Podocarpren).

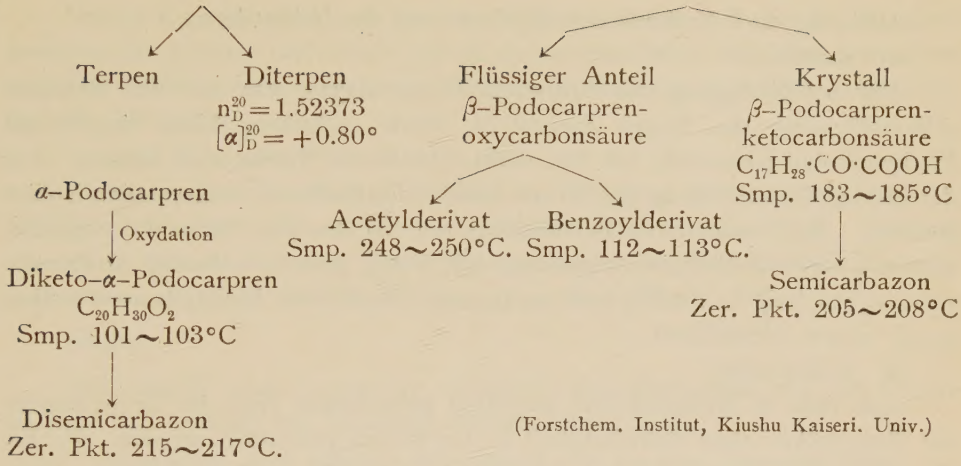
Von

Kitsuji Nishida und Hidetaka Uota

(Eingegangen am 26 Juni 1931)

Zur Konstitutionsaufklärung des *α*– und *β*–Podocarpren führten wir den oxdierenden Abbau der Diterpene (1) mit Kaliumpermanganatlösung (2) mit Braunstein und Schwefelsäure (3) mit Kaliumbichromat und Schwefelsäure (4) mit Kaliumpermaganatlösung bei Gegenwart von Alkali durch. Es wurde dabei (4) neben Terpen und carboxylierten Produkte, *β*–Podocarprenoxycarbonsäure, *β*–Podocarprenketocarbonsäure, noch Diketo–*α*–Podocarpren erhalten wie folgende Schema:





(Forstchem. Institut, Kiushu Kaiseri. Univ.)

Ueber die Kohlenhydrate aus den Samen von *Phaseolus Mungo* (Lu-tou).

Von

Syozi Miki

(Eingegangen June 30, 1931)

Die Gehalte an den Kohlenhydrate, angegeben in Prozenten der Trokensubstanz von Lu-tou, sind im folgenden:

1. Rohfaser	4.66%	4. Methylpentosan	Null
2. Pentosan	5.27%	5. Reduktionszucker	Null
3. Galaktan	1.03%	6. Totalle loesliche Kohlenhydrate (als Staerke)	66.02%

Loesliche Kohlenhydrate:

1. Die in heissem 95%igem Alkohol loeslichen Kohlenhydrate (als Glukose)	2.02%
2. Dextrin	3.40%
3. Staerke	57.00%
4. Hemicellulose und die anderen (als Galaktose)	3.03%

Ueber die in heissen 95%igem Alkohol loeslichen Kohlenhydrate.

Die mit Hilfe von Aether entfetteten, fein gepulverten Bohnen wurden mit heissem 95%igem Alkohol wiederholt ausgezogen. Die Beimischungen wurden mit Bleiessig, das Filtrat mit H_2S behandelt, dann wurden mit NH_3 neutralisiert und eingedunstet zum Sirup.

Durch die qualitativen Proben ist nachgewiesen worden, dass der dabei gewonnene Sirup, der Fehlingsche Loesung nicht reduziert, bei der Hydrolyse durch die verduennten anorganischen Saeure Glukose, Fruktose, Galaktose und Pentose (Xylose) lieferte. Dagegen liess sich keine Mannose nachweisen.

Isolierung und Nachweis der Raffinose und des Rohrzuckers.

a) Raffinose.

Der bei Zerlegung des Strontiumniederschlages, das aus der 80%igen Alkoholloesung des Sirups dargestellt wurde, erhaltene Sirup wurde mit Methylalkohol behandelt, um den darin enthaltenen Raffinose zu loesen. Aus der Methylalkoholloesung des Sirups konnte die Raffinose in Krystallen isoliert werden. Sie wurde durch Bestimmung der bei der Oxydation mit Salpetersaeure erhaltenen Schleimsaeuremenge (22.56%), ihres spezifischen Drehungsvermoegens ($[\alpha]_D^{20.5} = 104.8°$) und auch noch durch eine Krystallwasserbestimmung u. s. w. identifiziert.

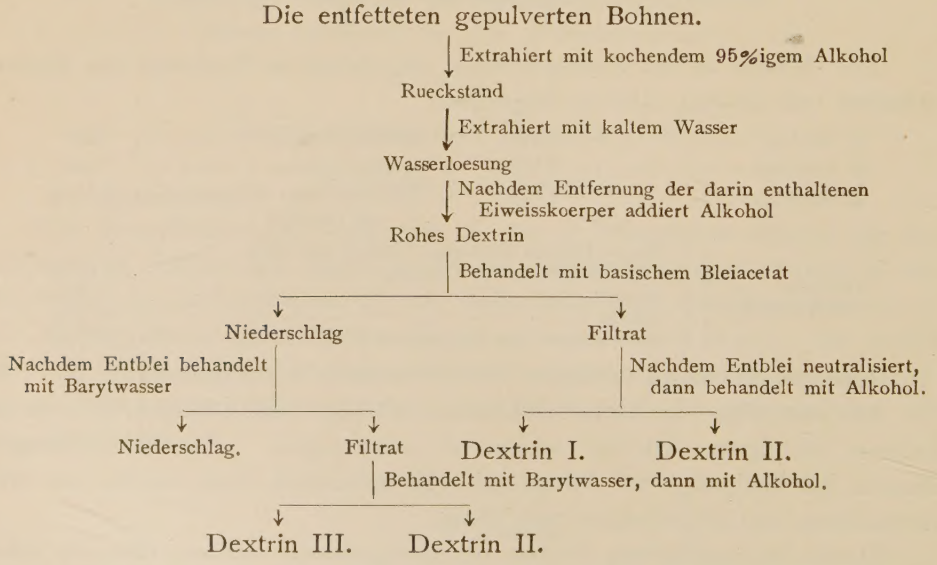
b) Rohrzucker.

Aus dem in Methylalkohol ungeloest gebliebenen Teile des Sirups konnte der Rohrzucker ohne Schwierigkeit in Krystallen gewonnen werden. Er hat die folgenden Eigenschaften: Monokline Krystalle. Schmelzpunkt 186°. $[\alpha]_D^{20.5} = 66.49°$.

Folglich ist wahrscheinlich, dass in den in heissem 95%igem Alkohol loeslichen Kohlenhydrate die Raffinose und der Rohrzucker vorhanden sind.

Ueber die in heissem 95%igem Alkohol unloelichen, in kaltem Wasser loeslichen Kohlenhydrate.

Nach folgendem Verfahren sind das Dextrin I, Dextrin II und Dextrin III isoliert worden.



Dextrin I.

Das Dextrin I ist nahezu identisch mit dem Amylodextrin von Lintner und Duell.

Einige Eigenschaften von Dextrin II sind aehnlich mit den des Erythrodextrins von Lintner und Duell, jedoch darauf man lieber das dabei gewonnene Dextrin II fuer ein Gemisch von Dextrin I und im folgenden erwaehntes Dextrin III halten.

Dextrin III.

Weisses, amorphes Pulver, loeslich in 70%igem Alkohol, unloeslich in 90%igem Alkohol. Jodreaktion rod. Reduktionsvermoegen Null. Bleiessig erzeugt in 5%iger Loesung einen Niederschlag; Barytwasser und Phosphowalframsaeure geben in 5%iger Loesung keine Faellung.

Staerke

Die Staerke von Lu-tou ist elliptisch (etwa 0.02～0.03 mm. im Durchmesser) und enthaelt die folgenden Nichtkohlenhydrate:

Asche 0.14% : P_2O_5 0.0675% : SiO_2 0.0641% : Die assozierten Fettsaeuren 0.60%.

Ueber die Verzuckerung der Staerke von Lu-tou.

Die Resultate der Bestimmung des durch Verzuckerung von Lu-toustaerke, Weizenstaerke, Maisstaerke, Kartoffelstarke, Glutinoese Reistaerke, Gewoehnliche Reisstarke und Kaoliangstaerke mit Taka-Diastase entstandenen Reduktionszuckers sind in der nachfolgenden Tabelle zusammengestellt.

Diastase-Wirkung Stunden	1	3.5	5	20
Kartoffelstaerke	1.4352g	1.7524	1.9448	2.2841
Lu-toustaerke	1.2808	1.6873	1.8093	2.2598
Kaoliangstaerke (Huan Nien)	1.2808	1.6645	1.7821	2.2370
Kaoliangstaerke (Hei Ko)	1.2683	1.6417	1.7294	2.1661
Weizenstaerke	1.2683	1.5533	1.6351	2.1661
Maisstaerke	1.2568	1.5256	1.6121	2.1430
Glutincese Reisstaerke	1.2568	1.5028	1.5891	2.1430
Gewcehnliche Reisstaerke	1.2455	1.4800	1.5659	2.1205

Wie man aus der Tabelle zu erschen vermag, ist die Verzuckerung von Lu-toustaerke mit Taka-Diastase besser als die anderen ausserdem Kartoffelstaerke.

Aber bei 3 stuendiger Verzuckerung der schon oben angegebenen verschiedenartigen Staerke mit 2.2%iger Salzsaeure im kochenden Wasserbade liefert Lu-toustaerke die groessere Glukosemenge als Glutinoese Reisstaerke, Maisstaerke und Kaoliangstaerke (Huan Nien) und die kleinere als die anderen.

Hemicellulosen.

Durch Anwendung von Norris und Preece-Methode (Bioch. J., **24**, 59, 1930), mittels welcher sie aus den Weizenkleien die verschiedenen Hemicellulosen herstellten, wurden die Hemicellulose I und Hemicellulose II aus

Lu-tou isoliert.

Hydrolyse der Hemicellulose I und Hemicellulose II mit Schwefelsaeure.

Sie geschah mit der 50-fachen Menge 3%iger Schwefelsaeure durch 4-stuendiges Erhitzen im kochenden Wasserbade und die mit Barytwasser entsaeuerten Fluessigkeiten wurden eingedampft zum Sirup.

Nachweis der Xylose in den Sirupe.

Aus den Sirupe der Hemicellulose I und Hemicellulose II wurden die Krystalle des Bertranschen Cadmiumbromxylonates erhalten, welches nach dem Umkrystallisieren Folgendes ergab.

$C_5H_9O_6CdBr+H_2O$	Ber.	Br	21.28,	Cd	29.94
I	Gef.	Br	21.35,	Cd	29.61
II	Gef.	Br	21.43,	Cd	29.70

Isolierung und Nachweis der Arabinose.

Durch Anwendung der von Maurenbrecher und Tollens (B., **39**, 3576, 1906) angegeben Methode der Trennung von Arabinose und Xylose auf diese Sirupe wurden die Krystalle des l-Arabinose-Diphenyl-Hydrazons (Schmelzp. 204°) erhalten. Durch die Zersetzung des dabei gewonnen Hydrazons mit Formaldehyd, wie Ruff und Ollendorf (B., **32**, 3236, 1899) angaben, ist Arabinose isoliert worden.

I $[\alpha]_D^{21°} = +103.8°$: II $[\alpha]_D^{21°} = +104.7°$

Nachweis der Galaktose.

Bei der Oxydation durch verduennte Salpetersaeure (S. G. 1.15) lieferten die beiden Sirupe Schleimsaeure (Schmelzp. 204°), welches Ca-Salz Folgendes ergab.

$CaC_6H_8O_8+1\,1/_2\,H_2O$	Ber.	CaO	20.38
I	Gef.	CaO	19.87
II	Gef.	CaO	19.92

Die Proben auf den anderen Zuckerarten.

Die bei der Hydrolyse von Hemicellulose I und Hemicellulose II mit Schwefelsaeure erhaltenen beiden Sirupe gaben die Tollenssche Naphtoresorcin-Reaktion. Dagegen hatten die Versuche zur Gewinnung von Mannosephenylhydrazon aus den Sirupen keinen Erfolg und die Pinoff-Schiwanoffsche Probe auf Ketosen gleichfalls eine negative Resultate gab.

Die in den beiden Hemicellulosen vorhandenen Mengen von Pentose-, Galktose-, und Uronsaeure-Gruppe sind in folgender Weise bestimmt:

Hemicellulosen	I	II
Ausbeute an Furfurol	40.80%	36.52%
Ausbeute an CO_2	2.30%	3.85%
Uronsaeure Anhydrid ($CO_2 \times 4$)	9.20%	15.40%
Furfurol aus Uronsaeure-Gruppe	1.54%	2.57%
Furfurol aus Pentose-Gruppe	39.26%	33.95%

Aus dem Angegebenen geht hervor, dass die Hemicellulose I aus 9.2% Uronsaeure Anhydrid, 73.4% Arabinose und Xylose (als Anhydroarabinose) und 17.4% Anhydrogalaktose und die Hemicellulose II aus 15.4% Uronsaeure Anhydrid, 63.5% Arabinose und Xylose (als Anhydroarabinose) und 21.1% Anhydrogalaktose besteht.

On the Chemical Properties of the Flavon-derivative Contained Tobacco Leaves with Special Referrence to the Colour and Qualities of Cured Leaves.

By

Hiroshi HASEGAWA

(Received August 6, 1931)

Summary.

1) The most important character for grading a cured tobacco leaf is its colour.

2) According to the traditional opinion the colouration of cured tabacco leaves is due to phlobaphene, which is derived from oxydation of tannine by the action of oxydase (O. Loew: Ztbl. Bakt. 2te Abt. VI. 108, 673, 1900).

3) It was now proved that a flavon-derivative is contained always in fresh tobacco leaves and also in nearly all kinds of cured tobacco leaves.

4) The flavon-derivative extracted from fresh tobacco leaves was identified with quercetin-rhamno-glucoside "Rutin ($C_{27}H_{32}O_{16}\cdot 2H_2O$)" on the ground of several physical and chemical properties.

5) The colour-change of the ordinary Japanese tobacco during its curing process may be divided practically into four stages, i. e.

1st : the drying leaves yet show a "greenish colour",
2nd: the leaves turn "yellowish" (yellowing stage),
3rd : the leaves become almost "brownish" (brownish stage),
4th : the leaves show a "nearly unchangeable brown colour" (fixed colour stage).

Microscopical observations upon the development and distribution of colouring matter in the leaf-cells from these four stages gave following results.

In the 1st stage the epidermal cells are colourless, but the palisade and inner parenchymatous cells are filled with chloroplasts. In the 2nd stage epidermal cells appear yet colourless, but the palisade and inner parenchymatous cells are losing their green colour and turning yellow, owing to the

carotinoid pigments, which remained after the decomposition of chlorophyll. Throughout the 3rd and 4th stages the inner tissues do not change their yellowish colour, while the reddish-brown colouring matter is found accumulating in the epidermal and adjacent cells though the mode of colour distribution can not be made out exactly in broken and collapsing tissue cells.

6) Cobaltpentamminchloride solution, which is known to easily oxidise Rutin, was allowed to act upon the fresh tobacco leaves inducing thereby the so-called "vitaloxydation". It was then observed that the colour of epidermal cells were changed yellow, red and brawnish successively, and the palisade and inner parenchymatous cells were at first greenish in colour, but gradually turned yellow. These colour changes, progressing slowly both in epidermis and inner cells, show much resemblances with those observed during the curing process. Moreover the above mentioned results indicated that the colouring matter is mostly localised in the epidermal cells. For detailed examination the epidermis stripped off from underlying tissue was immersed in a 1/100 mol. solution of cobalt-pentamminchloride and the gradual development of oxidation coloures, yellow to brown, was clearly observed under the microscope.

7) The above mentioned colour changes occuring in epidermal cells of tobacco (N. Tabacum, N. glauca) corresponds exactly to the oxdative reaction of Rutin caused by cobalt-pentamminchloride in test tube experiments. The epidermis of tobacco leaves become deep green upon the treatment with ferric chloride, the reaction also showing the presence of Rutin. On the other hand the leaf-epidermis of Pelargonium Zonale gives a blue reaction due to tannine. Thus it might be well concluded that the colouration of cured tobacco leaves is caused essentially by the oxidation product of Rutin contained in epidermal cells.

8) Quantitave determinations of the flavon-derivative in 233 kinds of the cured leaves by means of colorimetric and titration methods have shown in general that this substance is contained more abundantly in bright-coloured better leaves than in discoloured inferior ones, and also richly more in newer than in older leaves.

9) On the other hand any relation between tobacco tannine and the colour of cured leaves was not clearly indicated by means of ferric chloride and soda, except in the parts of vasculer bundles.

Biochemical Studies of *Salmonidae*. VII.

Studies on Synthetic Diets. I.

Effect of Chemical Composition of Diet on the Cultured Fish.

By

H. Sekine assisted by Y. Kakizaki

(Received September 14, 1931)

This investigation has been carried out to determine food values of the principal materials forming a synthetic diet for use in fish culture.

In dealing with this problem the first point to be decided was the amount of protein requisite for the purpose. To ascertain this three groups of trout-fry (*Salvelinus fontinalis Mitchill*) were kept for 8 weeks on three varieties of diet (Table I), containing 16%, 36% and 64% of protein respectively, the calorific value on the diet being the same in each case.

Table I.

Compositions of Diets.

	A	B	C
Meat residue	64	36	16
Starch	16	44	64
Lard	10	10	10
Osborne's salt	3	3	3
Oryzanin	1.5	1.5	1.5
Green leaves (fresh)	5	5	5

Feeding experiment. The results of these feeding experiments for 55 days (from July to September) at the Fishculture Station by the Lake of Kizaki, the Prefecture Nagano are summarized in Table II.

Table II.

Results of Feeding Experiments.

	A			B			C		
	Live weight	Number	Average weight	Live weight	Number	Average weight	Live weight	Number	Average weight
Initial	162	100	1.62	163	100	1.63	165	100	1.65
Final	108	39	2.77	139	49	2.84	235	92	2.55
Gain & loss	−54	−61	+1.15	−24	−51	+1.21	+70	− 8	+0.90
Missing	—	3	—	—	10	—	—	0	—
Large	41	8		44	8		136	47	
Medium	44.37	15		57.56	20		57.82	20	
Small	22.76	16		37.11	20		22.52 18.38	10 15	

According to the above data, it may be seen that the best result was obtained in the case of the fish fed on the lowest protein diet (16%) while those of the other two groups were not in such good condition, though some of them grew more quickly. This fact suggests that it may be useless some surplus protein which contain in a natural food (such as a small fish its nutritive ratio is about 1) and that at least any part of the protein consume for energy may be replaced by the material such as starch.

Chemical research. In the next place, the influence of these diets on the fish bodies fed on them was observed by the analytical results (Table III) of the animals servived and their diets. Analytical samples of fish in these groups were almost in the same average weight.

Table III.

Chemical Composition of the Analytical Samples fed on the three Diets having the Same Calorific Value.

	Live weight (mg.)	Solids	Ash	Crude fat	Protein N×6.25	N	Ca	Mg
A	2958	626.8	57.58	116.3	441.5	70.63	11.10	0.94
B	2878	650.0	61.16	135.5	443.9	71.03	11.23	0.91
C	2881	650.8	59.05	141.9	425.1	68.03	12.00	0.97
A	100	21.19	1.94	3.93	14.90	2.388	0.375	0.032
B	100	22.68	2.13	4.72	15.43	2.468	0.390	0.032
C	100	22.51	2.05	4.92	14.73	2.361	0.416	0.034
A		100	9.16	18.56	70.42	11.28	1.77	0.15
B		100	9.37	20.75	68.01	10.88	1.72	0.14
C		100	9.10	21.87	65.55	10.49	1.82	0.15

As regards the relation between the chemical composition of the fish and that of the diet, the quantity of protein and starch in the latter results directly in a relative amount of protein and fat in the fish in a dried state.

Conclusion

1. A synthetic diet as norrow (about 1.0) as nutritive ratio of a natural food such as an animal body may be not want in culture of fry of salmonoid fish, and that is some surplus protein consume for moving may be replaced by other energy producing substance like starch.

2. The quantity of dietary protein and starch effects directly on a relative amount of those (fat for starch) in the fish body.

Biochemical Studies on *Salmonidae*. VIII.

Studies on Synthetic Diets. II.

Coefficient, maximum value and economical maximum point of utilization of dietary protein.

By

H. Sekine

(Received September 14, 1931)

According to the results which had been worked out in the experiments reported in the former paper, compositions of diets may be due to different biological values of their contents, i. e. dietary protein, fat, carbohydrate minerals and etc..

In this paper, the writer have to consider further on the coefficient of the dietary protein which had been applied in the former study.

The coefficient in the different percentages of protein of real biological value (i. e. the proportion of absorbed nitrogen retained in a fish to the real applied nitrogen in the diet) are not equal, that is to say, as the results of the experiment, the diets which contain 16%, 36% and 64% of protein have been found to have the biological values 0.335, 0.204 and 0.109 respectively as shown in Tables I, II and III.

Table I.

Amounts of body protein accumulated by individual during the experiment.

		A			B			C		
		Initial	Final	Gain	Initial	Final	Gain	Initial	Final	Gain
Live weight	(mg.)	1620	2770	1150	1630	2840	1210	1650	2550	900
Body protein	(%)	12.9	14.9	—	12.9	15.4	—	12.9	14.8	—
Ditto weight	(mg.)	199	413	214	210	437	227	213	377	165

Table II.

Amounts of assimilated dietary protein during the experiment.

	A	B	C
Amount of diet per a fish per day (mg.)	80	80	80
Dietary protein %	64	36	16
Ditto per day per a fish (mg.)	51.2	28.8	12.8
Total protein for 55 days (mg)	2816	1580	704
Ditto accimilated (as 70%) (mg.)	1971	1109	493

Table III.

Coefficient of utilization of dietary protein.

	A	B	C
Accumulated protein in fish / Assimilated protein in fish	0.107	0.204	0.335

Now, the most provable relation between the protein contents in the diets and their coefficients are obtained as follows:

Contents of dietary protein (%) are 16, 36, 64..... x

Ditto coefficients are 0.335, 0.204, 0.109..... A

Natural logalithmic values of A..... .... y

Then taking x on holizontal axis, y on vertical axis x, y may be in a lineal relation.

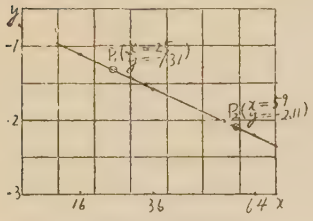


Fig. 1.

The equation of a straight line is

$$y+bx=a$$

which may be written $y=a-bx$ ...(1)

$$\log_e A=y=a-bx$$

$$\therefore \quad A=e^{a-bx} \quad \text{.....(2)}$$

Substituting the points of P_1 and P_2 (see Fig. 1) in the equation (1)

$$-1.31=a-b\times 25 \quad \text{.....(1')}$$

$$-2.11=a-b\times 59 \quad \text{.....(1'')}$$

Solving these equations simultaneously will give the values of a and b,

$$(1'')-(1')-0.8=-34b$$

$$\therefore \quad b=\frac{0.8}{34}=0.0235$$

Substituting the value of b in the equation (1')

$$\therefore \quad a=-1.31+0.59=-0.72$$

Therefore, the equation (2) may be written in the form

$$A=e^{-0.72-0.0235x} \quad \text{.....(3)}$$

Calculating the values of A from this equation, they are as shown in Table IV and the relation between x and A are shown in Fig. 2.

Table IV.

Values of A

x	A
10	0.385
20	0.303
30	0.240
40	0.190
50	0.150
60	0.118
70	0.093

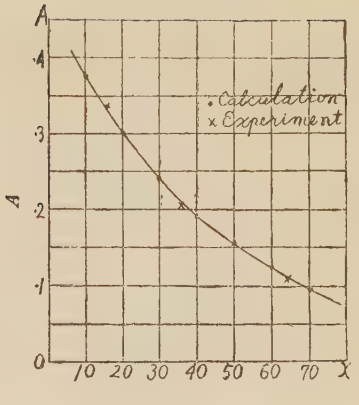


Fig. 2.

According to this fact, the coefficient of dietary protein may be descended with increasing its content (%).

Therefore, in the case where a diet applying the equal amount for keeping fish, the real utilization of the dietary protein is expressed by Ax, and from the equation given, as shown in Table V and Fig. 3. And the maximum

Table V.

Values of Ax

x	Ax
10	3.85
20	6.08
30	7.40
40	7.60
50	7.50
60	7.08
70	6.51

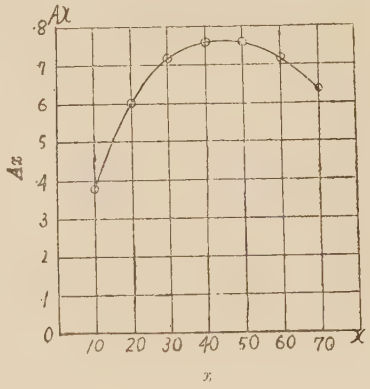


Fig. 3. Curve of Ax

value of Ax is obtained as follows:

$$Ax = xe^{-0.72-0.0235x} \qquad \frac{dAx}{dx} = e^{-0.72-0.0235x} - 0.0235xe^{-0.72-0.0235x}$$

$$e^{-0.72-0.0235x}(1-0.0235x)=0. \quad x=\frac{1}{0.0235}=42.5$$

Then the maximum value of Ax is in the case $x=42.5_5$.

From this result, it may be seen that in a synthetic diet the amount of protein content exceeding 43% goes to waste.

And the second time, the writer have something further to consider on an economical utilization of dietary protein. Although a coefficient of utilization of dietary protein is decreasing on the increasing of its amount, values of its

real utilization increse to the maximum point (42.5$_5$%), then a ratio of increase of Ax to that of x is not equal at every point, i. e. values of

$$\frac{Ax}{(Ax)_{max}}-\frac{x}{(Ax)_{max}} \quad \text{or} \quad \frac{Ax}{7.67}-\frac{x}{42.5_5}$$

increase, at first, gradually and then descending to zero at the end (Fig. 4 and Table VI). There is, therefore, the maximum point of this value at any amount of x, and on this point, the dietary protein should be most economically utilized (Fig. 5 and Table VII). The writer designated that point "Economical Maximum Point of Utilization of Dietary Protein".

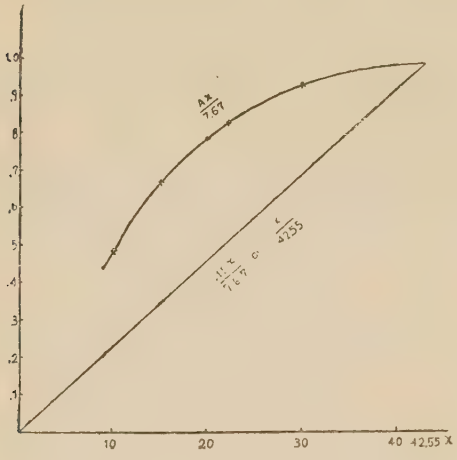


Fig. 4.

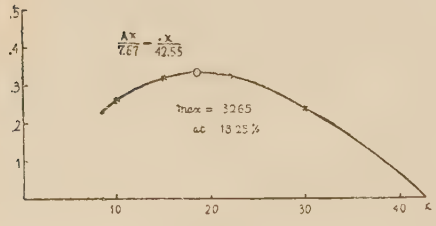


Fig. 5.

Table VI.

x	$\frac{Ax}{7.67}$	$\frac{x}{42.55}$
10	0.493	0.235
15	0.670	0.352+
20	0.793	0.469
22	0.830	0.517
30	0.935	0.705

Table VII.

x	$\frac{Ax}{7.67}-\frac{x}{42.55}$
10	0.257
15	0.317
16	0.319
17	0.324
18	0.326
19	0.324
20	0.323
30	0.231

This point can be obtained by the following solution:

$$\text{Put} \quad \frac{d}{dx}\left(\frac{Ax}{(Ax)_{max}}-\frac{ax}{(Ax)_{max}}\right)=\frac{d}{dx}\left(\frac{Ax}{7.67}-\frac{0.18x}{7.67}\right)$$

$$=\frac{e^{-0.72-0.0235x}-0.0235xe^{-0.72-0.0235e}}{7.67}-\frac{0.18}{7.67}=0$$

$$\text{Then} \quad e^{-0.72-0.0235x}(1-0.0235x)=0.18$$

$$\therefore \quad 1-0.0235x=0.18e^{0.72+0.0235x}$$

Then the maximum value of x is about 18 by graphical solution (Fig. 6 and Table VIII).

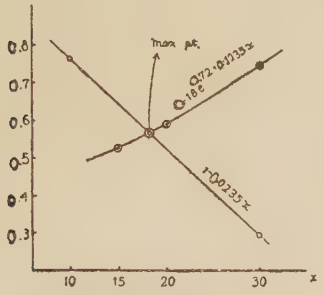


Fig. 6

Table VIII.

x	$1-0.0235x$	$0.18e^{0.72+0.0235x}$
10	0.765	—
15	0.644_5	0.525
20	0.530	0.593
30	0.295	0.747

Conclusion

Dietary protein (for use in trout fry culture) can be utilized for growth to 42.5% and about 18% of it, in case of dietary or supplied body or assimilated protein are of the same value, would be at an economical loss.

Summary

A coefficient of biological value of utilization (A) and an amount (x%) of dietary protein are in the relation as the following equation:

$$A=e^{-0.72-0.0235x}$$

A value of A is, therefore, gradually decreased with increasing of x.

Ax indicates a value of real utilization of dietary protein and it shows the maximum value when $x=42.5_5$%.

Then, although the value of utilization (Ax) should be ascended until $x=42.5$, a ratio of increase of Ax to that of x is not equal at every point, i. e. values of

$$\frac{Ax}{(Ax)_{\max}}-\frac{ax}{(Ax)_{\max}} \quad \text{or} \quad \frac{Ax}{7.67}-\frac{x}{42.5_5}$$

increase, at first, and then descend to zero on that point, and the maximum point of these values is in the case of $x\fallingdotseq 18$.

The author designated that point "Economical Maximum Point of Utilization of Dietary Protein".

From the results, an economical protein content of an artificial diet use in feeding trout-fry may be 20%～30%.

In conclusion I desire to express my thanks to Mr. Sato and Mr. Hoshino for their heepful abvice in this work.

Digestion Experiment of Soy Bean Cake and Kaoliang with Poultry.

By

Kozo Suzuki

(*Imperial Zootechnical Experiment Station, Chiba*)

(Received September 25, 1931)

1. Digestion Experiment of Soy Bean Cake.

Experimental Procedure.

The digestion experiment of soy bean cake was undertaken with two 2–years–old single comb white leghorn cocks. After a twenty–four hours' fast, they were dosed with 1 g. of thymol and a couple of hours later were given 10 c.c. of castol oil to remove the parasites in their intestines. Three days later, the birds were starved again for twenty–four hours and were performed the operation to produce an artificial anus by cutting the intestine at a point just back of where the urine emptied into it, and bringing the end out to the abdomen walls. After the operation, the health of the birds was carefully watched. When the operated parts were completely heald up, the cocks were fed with the basal ration, in which was mixed a small amount of soy bean cake ground fine enough to pass through a sieve with pores of 2.5 mm. in diameter. Day by day, the amount of the ground soy bean cake was increased and after five days the whole ration was changed to ground soy bean cake alone and for a few days more the birds were compelled to eat this food only. After a while the birds became accustomed to the apparatus of rubber bags which were held in place for collecting the urine and dung separately.

Before the collection of the dung was begun, the cocks were starved for fifteen hours, during which time they excreted all the dung in the intestines in first few hours. At the end of the fifteen hours past they were compelled to swallow 0.5 g. of charcoal which was suspended in about 10 c.c. of water. After four or five hours the birds excreted the charcoal alone. After a fifteen hours' starvation, a measured amount of ground soy bean cake was supplied in a vessel.

Twenty–four hours later, the food–vessel was taken away and the amount of food which remained was weighed to know the quantity of food consumption of twenty–four hours. Then, after four hours the birds were compelled again to swallow 0.5 g. of charcoal suspended in about 10 c.c. of water. After four or five hours the charcoal was excreted alone.

The amount of dung which was excreted in the time between the end

of the first excretion of charcoal and the begining of its second excretion represented exactly the undigested part of soy bean cake which has been taken in the experimental period of twenty-four hours.

Bird A took 139.0 g. of soy bean cake, and bird B 154.8 g. in the experimental period of twenty-four hours; bird A has excreted 247.2 g. of wet dung, and bird B 275.0 g.

Composition of Soy Bean Cake and the Dung.

		Moisture	Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Crude ash	Pure protein
		%	%	%	%	%	%	%	%
Air-dried state	Soy bean cake	13.640	80.860	43.563	7.298	3.950	26.049	5.500	42.440
	Dung of bird A	12.790	76.460	19.760	1.653	11.188	43.859	10.750	10.779
	Dung of bird B	12.575	76.335	18.189	1.800	11.845	44.501	11.090	11.901
Moisture-free state	Soy bean cake		93.631	50.443	8.451	4.574	30.163	6.369	49.143
	Dung of bird A		87.673	22.658	1.895	12.829	50.291	12.327	12.360
	Dung of bird B		87.315	20.805	2.059	13.549	50.902	12.685	13.613

Calculation of Digestibility Coefficient.

The 139.0 and 154.8 g. of soy bean cake which were taken by both birds can be converted into 120.0 and 133.7 g. of the moisture-free state, and 247.2 and 275.0 g. of wet dung were calculated to 43.0 and 46.3 g. of the moisture-free state respectively. Therefore, the digestibility coefficient of soy bean cake can be obtained as follows:

			Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Pure protein
Bird A	In dried soy bean cake 120.0 g.	(g.)	112.357	60.532	10.141	5.830	35.856	58.847
	In dried dung 43.0 g.	(g.)	37.699	9.743	0.815	5.637	21.504	5.266
	Digested quantity	(g.)	74.658	50.789	9.326	0.193	14.352	53.581
	Digestibility coefficient	(%)	66.447	83.904	91.963	3.310	40.027	91.051
Bird B	In dried soy bean cake 133.7 g.	(g.)	125.185	67.442	11.299	6.479	39.950	65.565
	In dried dung 46.3 g.	(g.)	40.427	9.633	0.953	6.403	23.438	6.255
	Digested quantity	(g.)	84.758	57.809	10.346	0.076	16.512	59.310
	Digestibility coefficient	(%)	67.652	85.717	91.566	1.173	41.332	90.460

Average Digestibility Coefficient of Soy Bean Cake.

Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Pure protein
67.0%	84.8%	91.8%	2.2%	40.7%	90.7%

2. Digestion Experiment of Kaoliang.

The digestion experiment of ground kaoliang was carried out with the

same cock (Bird A) and with the same procedure as the above mentioned experiment of the soy bean cake.

The cock took 231.0 g. of ground kaoliang in the experimental period of twenty-four hours and excreted 221.0 g. of wet dung.

Composition of Kaoliang and the Dung.

		Moisture	Organic matter	Curde protein	Crude fat	Crude fiber	Nitrogen-free extract	Crude ash	Pure protein
		%	%	%	%	%	%	%	%
Kaoliang	Air-dried state	12.425	85.425	11.228	3.590	1.340	69.262	2.155	10.689
	Dried state		97.539	12.821	4.099	1.530	79.089	2.461	12.206
Dung	Air-dried state	14.540	79.280	32.694	4.714	5.145	36.727	6.180	29.102
	Dried state		92.769	38.256	5.521	6.020	42.976	7.231	34.053

Culculation of Digestibility Coefficient.

The 231.0 g. of kaoliang can be converted into 202.3 g. of the moisture-free state and 221.0 g. of wet dung were calculated to 52.6 g. of the moisture-free state.

Digestibility coefficient of kaoliang is obtained as follows:

		Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen-free extract	Pure protein
In dried kaoliang 221.0 g.	(g.)	197.321	25.937	8.292	3.095	159.997	24.693
In dried dung 52.6 g.	(g.)	48.796	20.123	2.904	3.166	22.605	17.911
Digested quantity	(g.)	148.525	5.814	5.388	(−)0.071	137.392	6.782
Digestibility coefficient	(%)	75 3	22.4	65.0	—	85.9	27.5

Digestibility Coefficient of Kaoliang.

Organic matter	Crude protein	Crude fat	Nitrogen-free extract	Pure protein
75.3%	22.4%	65.0%	85.9%	27.5%

On the Identification of Phosphate-compounds in Soil.

By

S. Osugi, S. Yoshie and N. Nishigaki

(Received October 25, 1931)

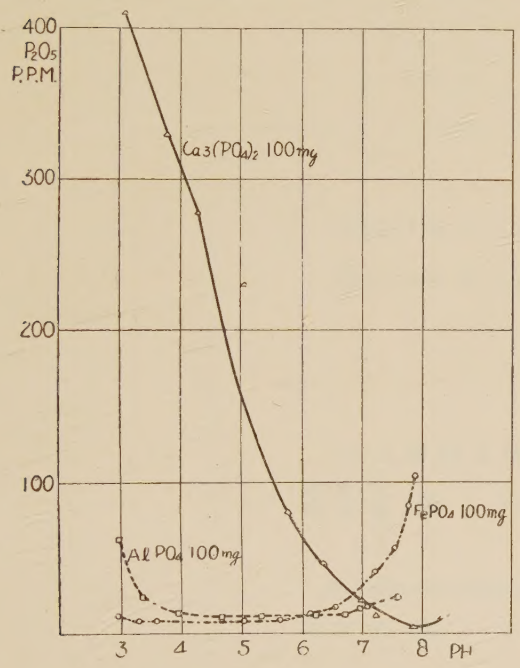
1. In order to identify soil-phosphates qualitatively and quantitatively by determining their solubility in solution with various pH, the writer tested at first the solubility of pure tricalcium phosphate, ferric phosphate and aluminium phosphate and obtained the following result (shown in the figure) which

shows that;

a. Tricalcium phosphate has minimum solubility at pH 7.68 which increases rapidly at more acid and a little at more alkaline side.

b. Aluminium phosphate dissolves least at pH between 4.07 and 6.93 and the solubility increases slowly both at more acid and alkaline sides.

c. Ferric phosphate shows minimum solubility at pH between 3 and 6 and it dissolves markedly at more alkaline side.



The characteristic of the above solubility curves was not changed by the amount of phosphate applied, by the co-existence of the above two or three salts and small quantity of soluble phosphate, although in the last case, larger solubility was noted at alkaline side for $Ca_3(PO_4)_2$, at acid side for $FePO_4$ and at all range of reaction for $AlPO_4$.

2. The writer made the same experiment with 11 soils and identified their phosphates by comparing the solubility curve with pure phosphates with the following result.

	phosphate identified	ratio of phosphate
4 soils	$FePO_4$	
2 soils	$Ca_3(PO_4)_2$ and $FePO_4$	1 : 1～1 : 2
3 soils	〃 〃	1 : 10～1 : 20
1 soil	$AlPO_4$ and $FePO_4$	1 : 10
1 soil	Same as above but with very small quantity of $Ca_3(PO_4)_2$	

3. It was also experimented that the change of monocalcium phosphate after various standing with soil, can be traced with the method.

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